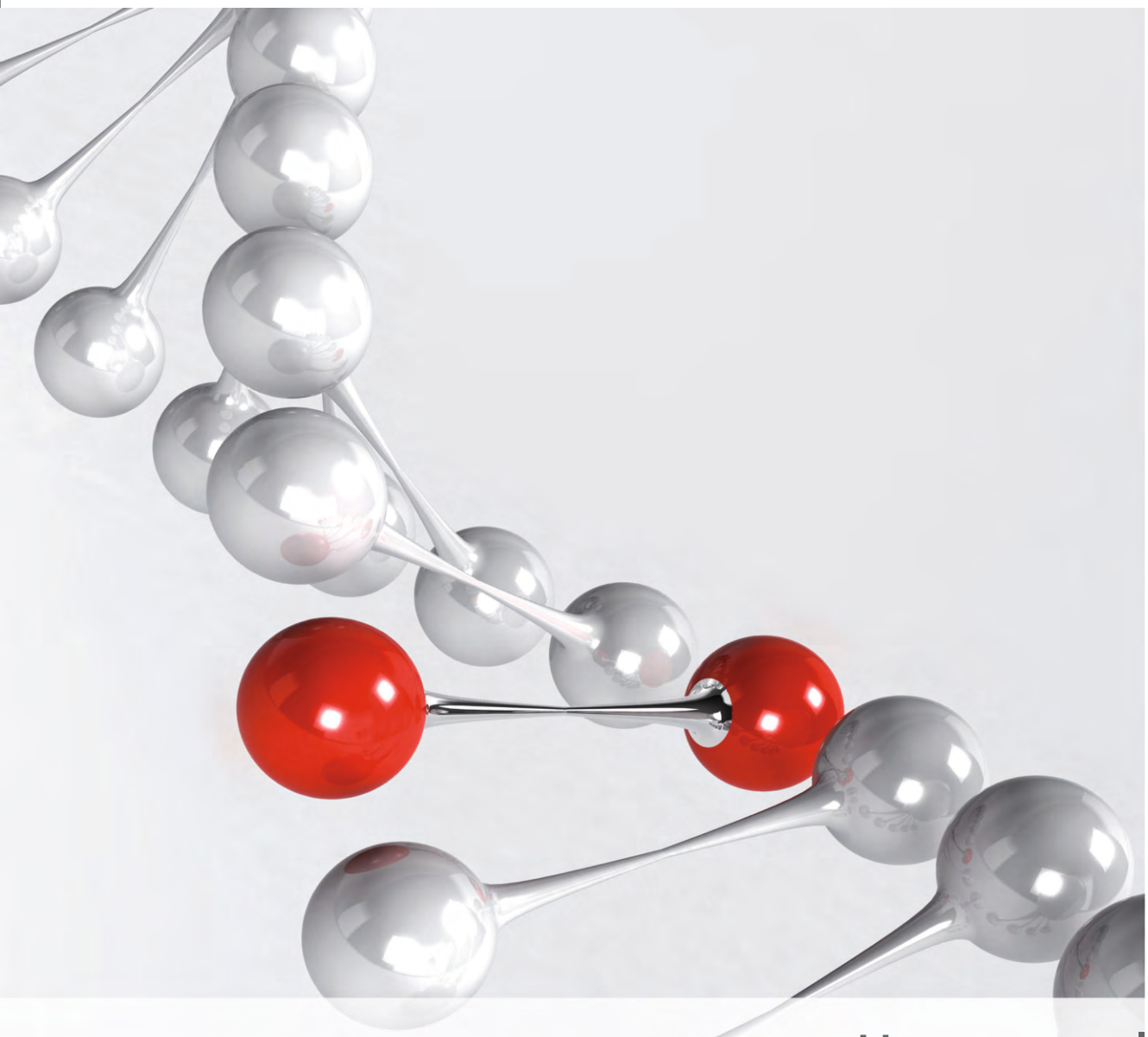
stratec
molecular



User manual
PSP® SalivaGene DNA Kit

for purification of total DNA from SalivaGene stabilized clinical swab (mouth brushes) and saliva sample

CE
IVD

REF 1035200x00

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for PSP® SalivaGene Systems

PSP® SalivaGene Systems are different tools to stabilize and purify genomic and bacterial DNA quantitatively from saliva and swabs (e.g. mouth brushes).
The **PSP® SalivaGene DNA Kit** in combination with the **SalivaGene® Buccal Swab,** the **SalivaGene Collection Module II, SalivaGene Collector** or the **SalivaGene Collector Premium** (SalivaGene® Collection Sets) is a modular system using a stabilization solution for collection, stabilization, storage and transportation of swab or saliva material without degradation of DNA for at least 12 month with a very efficient isolation method for high quality DNA.

The kit has been designed for isolation of genomic, mitochondrial and bacterial DNA for *in vitro* diagnostic analysis.

The kit is suitable neither for the isolation of DNA from stool samples or from fungi nor for purification of total RNA.

CE **IVD** Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for *in-vitro* diagnostic use in countries where the EU Directive 98/79/EC on *in vitro* medical devices is not recognized.

Trademarks: Invisorb®, PSP®, MSB®, , MSB ®, Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

PSP® is a registered trademark of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit content of the PSP® SalivaGene DNA Kit

Store dissolved Proteinase K at –20°C!
Store all other kit components at room temperature (RT)!

	5 extractions	50 extractions	250 extractions
Catalogue Number	1035200100	1035200200	1035200300
Binding Buffer A	2 x 1 ml (ready to use)	4 ml (final volume 15 ml)	2 x 9 ml (final volume 2 x 30 ml)
Proteinase K	for 250 µl working solution	for 1.1 ml working solution	for 5 x 1.1 ml working solution
Wash Buffer SL	15 ml (ready to use)	24 ml (final volume 60 ml)	1 x 80 ml (final volume 200 ml)
Elution Buffer	2 ml	15 ml	30 ml
RTA Spin Filter Set	5	50	5 x 50
RTA Receiver Tubes	5	50	5 x 50
1.5 ml Receiver Tubes	5	50	5 x 50
Manual	1	1	1
Initial steps	Dilute **Proteinase K** with 250 µl ddH_2O. Mix thoroughly until completely dissolving and store at -20°C! Prepare 10 ml of **70% Ethanol.** Incubate the needed amount of **Elution Buffer** at 48°C.	Add 11 ml 99.7% Isopropanol to the bottle **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Dilute **Proteinase K** with 1.1 ml ddH_2O. Mix thoroughly until completely dissolving and store at -20°C! Add 36 ml of 96-100% Ethanol to the bottle **Wash Buffer SL**, mix thoroughly and store with tightly closed cap. Prepare 100 ml of **70% Ethanol.** Incubate the needed amount of **Elution Buffer** at 48°C	Add 21 ml 99.7% Isopropanol to each bottle **Binding Buffer A**. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Dilute each tube **Proteinase K** with 1.1 ml ddH_2O. Mix thoroughly until completely dissolving and store at -20°C! Add 120 ml of 96-100% Ethanol to the bottle **Wash Buffer SL**, mix thoroughly and store with tightly closed cap. Prepare 500 ml of **70% Ethanol.** Incubate the needed amount of **Elution Buffer** at 48°C

Content of SalivaGene® Collection Sets

SalivaGene® Collection Sets	50 pieces	8 x 50 pieces
SalivaGene® Buccal Swab	1035230200	1035230300
SalivaGene® Collection Module II	1035212200	1035212300
SalivaGene® Collector Premium	1035210200	
SalivaGene® Collector	1035211200	

Symbols

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit components, except the dissolved Proteinase K of the **PSP® SalivaGene DNA Kit** should be stored well sealed and dry at room temperature and are stable for at least 12 months under these conditions. Room temperature (RT) is defined as range from 15 - 30°C.

Dissolved **Proteinase K** must be stored at -20 °C. Repeated freezing and thawing will reduce the activity of **Proteinase K** dramatically. Dividing of **Proteinase K** into aliquots and storage at -20°C is recommended.

Binding Buffer A charged with Isopropanol, **Wash Buffer SL** charged with Ethanol should be appropriate sealed.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by warming carefully.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **PSP® SalivaGene DNA Kit** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.
STRATEC Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **PSP® SalivaGene DNA Kit**

have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.
If you have any questions or problems regarding any aspects of **PSP® SalivaGene DNA Kit** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany **+49-(0)30-9489-2901/ 2910**
from abroad **+49-(0)30-9489-2903/ 2907**
or contact your local distributor.

Intended use of the PSP® SalivaGene DNA Kit

The **PSP® SalivaGene DNA Kit** is suitable for reproducible purification of total DNA from 500 µl stabilization media of swab and saliva samples in forensic, human-identity, genetic, biosecurity and pathogen and for *in vitro* diagnostic analyses. The purification is very efficient and the isolated DNA performs well in downstream applications, such as quantitative PCR and STR analysis, with high signal-to-noise ratios.

The system consists four modules, three collection sets and one extraction module, useable independently or in combination. Also included is an easy to handle protocol for DNA purification by precipitation.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from serum, plasma, fungi nor from parasites or isolation and purification of total RNA.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications. STRATEC Molecular Products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described

herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The Product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular Product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **PSP® SalivaGene DNA Kit** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **PSP® SalivaGene DNA Kit** and for the **SalivaGene® Stabilization Sets** to which they apply are listed below as follows:.

Proteinase K

danger
H315-319-334-335 P280-305-351-338-310-405

Saliva DNA Stabilizer

danger
H319 P305-351-338

H319:	Causes serious eye irritation.
H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
H315:	Causes skin irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P273:	Avoid release to the environment.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA:	**1 – 352 – 323 – 3500**
in USA :	**1 – 800 – 535 – 5053**

Product characteristic of the PSP® SalivaGene DNA Kit

Starting material	Yield	Time	Ratio
500 µl of stabilization buffer (SalivaGene DNA Stabilizer) for all 3 Saliva Collection Sets	up to 10 µg (depends of kind of starting material)	30 min	A_{260} : A_{280} 1.7 – 2.0
500 µl of swab stabilization (SalivaGene DNA Stabilizer)	up to 1 µg (depends on the donor)		

The **PSP® SalivaGene DNA Kit** uses the cost efficient **PSP® Technology** (Preanalytical-Sample-Processing). It is a modular designed system for collection, stabilization, storage and transportation of saliva or swab samples completed with DNA purification. The system combines the use of prefilled **SalivaGene® Collection Sets** for saliva or swab sample collection with the storage and stabilization of respective specimen. The DNA is protected during transportation. Bacterial samples are prelysed, bacterial growth is inhibited. The procedure is completed by a very efficient and fast isolation method (up to 500 µl, about 30 min) which results in high quality total DNA. This kit has been designed for isolation of DNA from host organism, as well as for DNA from pathogenic bacteria and eukaryotes for *in vitro* diagnostic analysis.

After sample collection the saliva or swab sample it is transferred (as described), in one of the available **SalivaGene® Collection Sets**, prefilled with **SalivaGene® DNA Stabilizer**. The raw material is lysed under specific conditions. The **SalivaGene® DNA Stabilizer** effects inactivation of DNases and prevents degradation of DNA, it preserves the microorganism titre and prelyses bacteria. The stabilized sample may be shipped in the **SalivaGene® DNA Stabilizer** containing tubes during 12 month at ambient temperature.

The sample may be stored under this buffer at -20° for a several years. For the DNA extraction an aliquot may be used, the residual sample is stored for further extractions.

500 µl aliquots of the sample are used in the DNA isolation procedure. At first the proteins are degraded with Proteinase K, followed by a binding of the DNA to a Spin Filter while contaminants pass this filter. The DNA is eluted in either water or Elution Buffer provided with the kit.

Yields are from sample to sample depending on factors as the nature of the sample (swab or saliva) and the DNA contents of the respective samples.

The Kit procedure requires neither phenol/ chloroform extraction nor alcohol precipitation. A minimal interaction by the user allows safe handling of potentially infectious samples. The whole process is designed to avoid sample-to-sample cross-contamination.

Due to the high purity, the isolated genomic DNA is ready to use for a broad panel of downstream applications (see below) or can be stored at –20°C for subsequent use

- PCR*, Real Time PCR Analysis*, RFLP / AFLP*- Analysis
- Restriction Enzyme Digestion
- HLA-Typing
- Pathogen Detection

For an higher yield of DNA use our easy to handle **PSP® SalivaGene Precipitation Kit** (order no. 1035290200)

*) The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the **PSP® SalivaGene DNA Kit** cannot be construed as an authorization or implicit licence to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Sampling and sample storage

Starting material

The amount of starting material used in PSP® SalivaGene DNA Kit procedures can vary slightly, depending on the amount of DNA present in the sample. Specific guidance for starting amounts is given in the individual protocols.

Swab, Saliva: The protocol works with aliquots of transport media for collection of fresh saliva or swab samples. The DNA is stable for at least 12 month at room temperature in the transport media (**SalivaGene® DNA Stabilizer**). The aliquots of the stabilization buffer may be processed on the same day as collection or stored for future processing. Long-term storage of the sample should be done at - 20°C.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Principle and procedure

The **PSP® SalivaGene DNA Kit** procedure comprises following steps:

- collection of the material using one of the **SalivaGene® Collection Sets**
- lysis of cells under non-chaotropic conditions at elevated temperature in the Stabilization Buffer (**SalivaGene® DNA Stabilizer**)
- protein digestion
- binding genomic DNA to the membrane of a RTA Spin Filter
- washing the membrane and elimination of contaminants and ethanol
- elution of pure genomic DNA

This manual contains 2 protocols, according to the different requirements of the starting materials.

Protein digestion
Proteins will be digested in the lysed samples in the presence of **Proteinase K**.

Binding genomic DNA
By adding **Binding Buffer A** to the lysate, optimal binding conditions will be adjusted. Each lysate is then applied to an RTA® Spin Filter and genomic DNA is adsorbed to the membrane.

Removing residual contaminations
Contaminants are efficiently washed away using **Wash Buffer SL** and **70% Ethanol**, while the total DNA remains bound to the membrane.

Elution of pure genomic DNA
Genomic DNA is eluted from the Spin Filter using 50 - 100 µl **Elution Buffer**.
The eluted DNA is ready for use in different downstream applications.

Yield and quality of DNA

The amount of purified DNA in the **PSP® SalivaGene DNA Kit** procedure from different samples depends on the sample type and sample source. Yield and quality of isolated DNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed according to manufacturer's specifications.

Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipette tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard contaminated gloves.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar airflow until the samples are lysed.
- This kit should only be used by trained personnel.

Preparing reagents and buffers

Before starting a run, bring all reagents to room temperature. Where necessary, gently mix and re-dissolve any precipitates by incubation at 30°C. Swirl gently to avoid foaming.

1. Adjust the Thermomixer to 48°C.
2. Warm up the needed amount of **Elution Buffer** to 48°C
   (up to 100 µl **Elution Buffer** are needed per sample).
3. Label the needed amount of RTA Spin Filter (lid).
4. Label the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube).
5. Add the needed µl ddH_2O to reaction tube with **Proteinase K** (see below). Vortex for 5 s
6. Add the needed amount of Isopropanol to the **Binding Buffer A**. Vortex for 5 s
7. Add the needed amount of Ethanol to the **Wash Buffer SL**. Vortex for 5 s
8. Prepare the needed amount **of 70% Ethanol**.

5 DNA extractions:
Add 250 µl ddH_2O to **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C! **Binding Buffer A** and **Wash Buffer SL** are ready to use! Prepare 10 ml of **70% Ethanol**.
50 DNA extractions:
Add 11 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Dilute the **Proteinase K** with 1.1 ml ddH_2O. Mix thoroughly until completely dissolving and store at -20°C! Add 36 ml of 96 - 100 % Ethanol to **Wash Buffer SL** mix thoroughly and keep the bottle always firmly closed. Prepare 100 ml of **70% Ethanol**.
250 DNA extractions:
Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Dilute each tube **Proteinase K** with 1.1 ml ddH_2O. Mix thoroughly until completely dissolving and store at -20°C! Add 120 ml of 96 - 100 % Ethanol to **Wash Buffer SL** mix thoroughly and keep the bottle always firmly closed. Prepare 500 ml of **70% Ethanol**.

Reagents and equipment to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** under each STRATEC Molecular kit and kit component.

- Microcentrifuge
- Thermomixer (for 48°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortexer
- Reaction tubes (1.5 ml or 2.0 ml)
- dd H_2O
- 96 - 100 % Ethanol
- Isopropanol *

*The **PSP® SalivaGene DNA Kit** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Ordering No. 6752

Applichem
2-Propanol für die Molekularbiologie
Ordering No. A3928

Sigma
2-Propanol
Ordering No. 59304-1L-F

Important indications

1. Process only as many samples as the microcentrifuge allows to process.

2. Sample and buffers should be thoroughly mixed and should have room temperature

3. The elution can be done by using lower amount of **Elution Buffer**. This may result in a higher concentration of DNA. But pay attention that minimum volume for elution is 30 µl, but this will reduce the yield. Elution volume between 2 x 50 µl up to 200 µl will realize comparable results.

4. The eluted DNA volume can be lower than the added **Elution Buffer** volume. **Elution Buffer** should be preheated to 48 °C.

5. **Elution Buffer** doesn't contain EDTA.

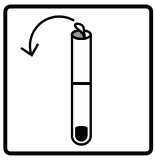
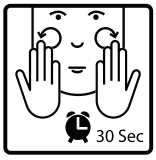
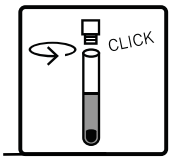
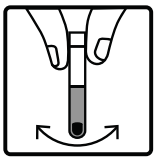
6. The yield can be increased, if the incubation time with preheated **Elution Buffer** will be prolonged.

PSP®-Treatment with SalivaGene® Collector and Collector *Premium*

Intended Use: Collection of human saliva samples for DNA stabilization and extraction. For research use only.
The DNA in SalivaGene stabilized samples is stable for 1 year at room temperature within the expiry date. The product is intended for use under supervision of professionals only, such as technicians, physicians and biologist trained in molecular biological techniques.

Contents: SalivaGene funnel, lid, tube, each tube contains approx. 150 mg of SalivaGene reagent.

Limitations of the procedure: Do not eat, drink, smoke or chew gum at least 30 min prior to saliva collection. Collecting 2 ml of saliva may require several minutes. Putting some grains of sugar on the tongue helps to produce saliva and does not interfere with the stabilization.

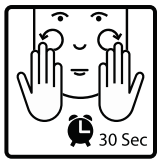
1. 	Remove seal from tube completely, and discard the seal or unscrew lid from tube and put it aside for further use.
2.	Insert funnel into tube tightly.
3. 	Rub cheeks against teeth intensely for 30 sec.
4.	Collect saliva to indicated fill level, avoid making and measuring air bubbles.
5. 	Remove and discard the funnel. Press lid firmly on the tube until it clicks or screw lid tightly onto the tube again.
6. 	Gently shake tube for 15 sec to dissolve white reagent.
7.	Store the tube upright for 2-20 min with occasional shaking until SalivaGene reagent is dissolved. Some cloudy material may occur during this process. This does not interfere with stabilization.

PSP®-Treatment with SalivaGene® Collection Module II

Intended Use: Collection of human saliva samples for DNA stabilization and extraction (using the SalivaGene DNA Extraction Kits).
The DNA in SalivaGene stabilized samples is stable for 1 year at room temperature within the expiry date. The product is intended for use under supervision of professionals only, such as technicians, physicians and biologist trained in molecular biological techniques.

Contents: Collection tube, Stabilizer tube. The stabilizer tube contains approx. 2 ml of liquid Saliva DNA Stabilizer reagent.

Limitations of the procedure: Do not eat, drink, smoke or chew gum at least 30 min prior to saliva collection. Collecting 2 ml of saliva may require several minutes. Putting some grains of sugar on the tongue helps to produce saliva and does not interfere with the stabilization.

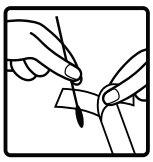
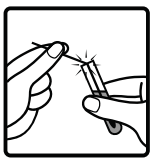
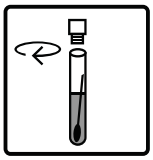
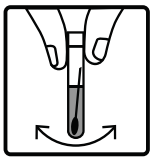
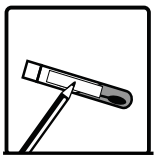
1.	Unscrew lid from Collection tube and put it aside for further use.
2. 	Rub cheeks against teeth intensely for 30 sec.
3.	Collect saliva to indicated fill level, avoid making and measuring air bubbles.
4.	Unscrew and discard lid from Stabilizer tube. Pour Saliva DNA Stabilizer reagent into Collection tube.
5.	Screw lid tightly onto the Collection tube again.
6.	Shake tube for 15 sec to mix saliva and Saliva DNA Stabilizer reagent.

PSP®-Treatment of SalivaGene® Buccal Swab

Intended Use: Collection of human oral samples for DNA stabilization and extraction (using the SalivaGene DNA Extraction Kits).
The DNA in SalivaGene stabilized samples is stable for 1 year at room temperature within the expiry date. The product is intended for use under supervision of professionals only, such as technicians, physicians and biologist trained in molecular biological techniques.

Contents: Swab, Stabilizer tube, label for donor description. The Stabilizer tube contains approx. 2 ml of liquid DNA Stabilizer reagent.

Limitations of the procedure: Do not eat, drink, smoke or chew gum at least 30 min prior to swab collection. Ensure the swab tip does not come into contact with any surface prior to collection. Put the Stabilizer tube upright to prevent the liquid inside the tube from spilling. Be sure to move the swab over the entire cheek and to moisten it with saliva.

1. 	Open swab package and remove swab without touching the tip.
2.	Insert swab into the mouth and rub swab tip firmly against the inner cheeks for about 30 seconds on each side.
3.	Unscrew lid from Stabilizer tube and put it aside for further use. Insert swab into the Stabilizer tube.
4. 	Break swab tip at breaking point. Discard the broken handle part.
5. 	Screw lid tightly onto the Stabilizer tube again.
6. 	Gently shake tube for 15 sec to mix buccal cells and DNA Stabilizer reagent.
7. 	Label Stabilizer tube with donor name and collection date using provided label.

Scheme of the PSP® SalivaGene DNA Kit

Please read protocols prior the start of the preparation carefully

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Transfer 500 µl aliquots of the stabilized sample from the **SalivaGene® Collection Sets** to a 1.5 ml Reaction Tube (not provided).

1. Protein removal

Add 20 µl of **Proteinase K**.
Incubate for 20 minutes at 48°C under continuously shaking.

2. Realizing optimal binding conditions

Add 200 µl of **Binding Buffer A** *(follow preparing instructions).*
Mix all completely by vortexing.

3. Binding of the DNA

Transfer the whole suspension onto a RTA Spin Filter Set.
Incubate for 1 minute at RT.
Centrifuge for 2 minutes at 11.000 x g (11.000 rpm).
Take the Spin Filter out of the RTA Receiver Tube and discard the RTA Receiver Tube

4. Washing of the Spin Filter

Place the Spin Filter into a new RTA Receiver Tube and add 600 µl **Wash Buffer SL** to the Filter. Centrifuge at 11.000 x g (11.000 rpm) for 1 minute. Discard filtrate.

Add 700 µl **70% Ethanol** to the Filter. Centrifuge at 11.000 x g (11.000 rpm) for 1 minute. Discard filtrate.

Repeat the second washing step.
Discard filtrate.

Remove ethanol by centrifugation for 4 minutes at maximum speed.

5. Elution of DNA

Place the RTA Spin Filter into a 1.5 ml Receiver Tube and add 50 –100 µl of prewarmed **Elution Buffer**. Incubate for 1 minute at room temperature. Close the Spin Filter and centrifuge for 1 minute at 11.000 x g (11.000 rpm).

Protocol I: Human DNA extraction from stabilized material by the PSP® SalivaGene DNA Kit

Please read the instructions carefully and conduct the prepared procedure.

Important *Transfer the needed amount of **Elution Buffer** into 2.0 ml Receiver Tube (not included in the kit) and place the tube at 48°C.*

Attention: *Please be aware, that you have to prepare the **Binding Buffer A** – see instruction page: 9*

1. Protein removal

Transfer 500 µl of **Sample/DNA Stabilizing Solution** from the **SalivaGene® Collection Sets** into a 1.5 ml reaction tube (not provided).

Add 20 µl of **Proteinase K** and incubate for 20 min at 48°C under continuously shaking or every 5 minutes flicking the tube.

Note: *If you have to archive a swab sample e.g. for forensic purposes, please use only 250 µl of the sample for preparation. In this case you add 250 µl of water to the stabilization solution and continue with the protocol as follows.*
If you have to archive a SalivaGene sample e.g. for forensic purposes, just freeze the rest you don't use for preparation. The rest of the stabilized sample can be stored at –20°C for years.

2. Binding of DNA to the RTA Spin Filter

Add 200 µl of **Binding Buffer A** to the 1.5 ml reaction tube, mix well. Transfer the suspension onto a RTA Spin Filter Set. Wait for 1 minute, then centrifuge for 2 minutes at 11.000 x g (11.000 rpm). Take the Spin Filter out of the Receiver Tube and discard the Receiver Tube.

3. First washing of the RTA Spin Filter

Place the Spin Filter into a new 2.0 ml Receiver Tube and add 600 µl **Wash Buffer SL** to the Filter. Centrifuge at 11.000 x g (11.000 rpm) for 1 minutes. Take the Filter from the Receiver Tube, discard the filtrate.

4. Second washing of the RTA Spin Filter

Place the Spin Filter back into the 2.0 ml Receiver Tube and add 700 µl **70% Ethanol** to the Filter. Centrifuge at 11.000 x g (11.000 rpm) for 1 minute. Take the Filter from the Receiver Tube, discard the filtrate and then place the Spin Filter again into the Receiver Tube. Repeat the washing step. Discard the filtrate again and put the Spin Filter back into the Receiver Tube. Close the Tube and centrifuge for 4 min at maximum speed (for complete removal of ethanol).

5. Elution of DNA

Place the Spin Filter into a new 1.5 ml Receiver Tube and add 50 –100 µl of prewarmed **Elution Buffer**. Incubate for 1 min at room temperature. Close the Spin Filter and centrifuge for 1 min at 11.000 x g (11.000 rpm).
Discard the Spin Filter. The filtrate contains the pure DNA.

Note: *The DNA can also be eluted with a lower volume of **Elution Buffer**. It is also possible to do the elution step two times with equal volumes of **Elution Buffer**. These will lead to slightly increased total yield. But pay attention, that minimum volume for the elution is 30 µl.*

Protocol II: Bacterial DNA extraction from stabilized material by the PSP® SalivaGene DNA Kit

Please read the instructions carefully and conduct the prepared procedure.

Important *Transfer the needed amount of **Elution Buffer** into 2.0 ml Receiver Tube (not included in the kit) and place the tube at 48°C.*

Important: ***Bacterial swabs must be taken by medical staff.***

Attention: *Please be aware, that you have to prepare the **Binding Buffer A** – see instruction page: 9*

1. Protein removal

Transfer 500 µl of **Sample/DNA Stabilizing Solution** from the **SalivaGene® Collection Sets** into a 1.5 ml reaction tube (not provided).

Add 10 µl of **Lysozyme** (10 mg/ ml)* and incubate for 10 min at 37°C under continuously shaking or every 5 minute flicking the tube. Add 20 µl of **Proteinase K** and incubate for 20 min at 48°C with shaking or every 5 minute flicking of the tube. Incubate the sample for additional 10 minutes under continuously shaking at 95°C. This step will lead to a thermic disintegration of bacterial cell wall structures. Human Genomic DNA of the cellular material might be broken by this step!

2. Binding of DNA to the RTA Spin Filter

Add 200 µl of **Binding Buffer A** to the 1.5 ml reaction tube, mix well. Transfer the suspension onto a RTA Spin Filter Set. Wait for 1 minute. Then centrifuge for 2 minutes at 11.000 x g (11.000 rpm). Take the Spin Filter out of the Receiver Tube and discard the Receiver Tube.

3. First washing of the RTA Spin Filter

Place the Spin Filter into a new 2.0 ml Receiver Tube and add 600 µl **Wash Buffer SL** to the Filter. Centrifuge at 11.000 x g (11.000 rpm) for 1 minute. Take the Filter from the Receiver Tube, discard the filtrate.

4. Second washing of the RTA Spin Filter

Place the Spin Filter back into the 2.0 ml Receiver Tube and add 700 µl **70% Ethanol** to the Filter. Centrifuge 11.000 x g (11.000 rpm) for 1 minute. Take the Filter from the Receiver Tube, discard the filtrate and then place the Spin Filter again into the Receiver Tube. Repeat the washing step. Discard the filtrate again and put the Spin Filter back into the Receiver Tube. Close the Tube and centrifuge for 4 min at maximum speed (for complete removal of ethanol).

5. Elution of DNA

Place the Spin Filter into a new 1.5 ml Receiver Tube and add 50 –100 µl of prewarmed **Elution Buffer**. Incubate for 1 min at room temperature. Close the Spin Filter and centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the Spin Filter. The filtrate contains the pure DNA.

Note: *The DNA can also be eluted with a lower volume of **Elution Buffer**. It is also possible to do the elution step two times with equal volumes of **Elution Buffer**. These will lead to slightly increased total yield. But pay attention, that minimum volume for the elution is 30 µl.*

for gram-positive bacteria, not included in the kit

Troubleshooting

Problem	Cause	Comments and suggestions
low amount of DNA	insufficient cell lysis	increase lysis time , reduce amount of starting material overloading of Spin Filter reduces yield
	insufficient cell lysis due to decreased **Proteinase K** activity	repeat the DNA purification procedure with a new sample and freshly prepared **Proteinase K** stock solution. Be sure to store the stock solution at –20°C.
	insufficient lysis time	the DNA purification procedure should be done not earlier than 1 hour after sampling
	inefficient binding of DNA to the membrane e.g. due to insufficient mixing with **Binding Buffer A**	overloading RTA Spin Filter reduces yield use correct amount of **Binding Buffer A** mix sample **with Binding Buffer A** by pipetting up and down 4-5 times or by vortexing (5 sec) prior to transfer the sample onto filter
	low percentage alcohol used	repeat purification procedure with a new sample
	incomplete elution	increase incubation time with preheated **Elution Buffer** to 5 - 10 min , elute twice with each 100 µl **Elution Buffer** use higher volume of Elution Buffer.
	low sample DNA-concentration	elute the DNA with lower volume of **Elution Buffer**
	pH of water incorrect (acidic)	low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use **Elution Buffer (**contains only 10 mM Tris – HCL, no EDTA)
degraded or sheared DNA	old material with several freeze thaw cycles	avoid repeated thawing and freezing of the material
clogged Spin Filter	insufficient lysis	perform isolation as described in protocols
	too much starting material	increase lysis time with lysis buffer **SalivaGene® DNA Stabilize** increase centrifugation time and/or speed reduce amount of starting material
problems with subsequent applications (e.g. in PCR)	ethanol in the eluted DNA	verify if the recommended centrifugation time was reached increase centrifugation time for the elimination of ethanol if necessary
	salt in the eluate	**Wash Buffer** should be stored and used at RT verify **Wash Buffer** on the precipitation of salt. If there are precipitations dissolve this by careful warming up to 30°C
	reduced sensitivity of amplification reaction	adjust the volume of eluate added as template in the amplification reaction
A_{260}/A_{280} ratio for purified DNA is low	insufficient cell lysis and protein degradation due to decreased **Proteinase K** activity	see above under " low amount of DNA"
	insufficient lysis due to insufficient **Proteinase K** activity	see above under " low amount of DNA"

Appendix

General notes on handling DNA

Starting material
This kit is designed for extraction of DNA from saliva and swabs. These materials show big variation in DNA contents. The purification of some apoptotic DNA is normal.

Nature of DNA
The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, and long-template PCR.

Storage of DNA
A working stock of DNA can be stored at 2 – 4˚C for several weeks. For long term storage DNA should be stored at -20˚C, but storing at – 20°C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Drying, dissolving and pipetting DNA
Avoid over drying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

Ordering information

Product	Package Size	Catalogue No.
PSP® SalivaGene DNA Kit	5 preparations	1035200100
PSP® SalivaGene DNA Kit	50 preparations	1035200200
PSP® SalivaGene DNA Kit	250 preparations	1035200300
SalivaGene® Collector Premium	5 pieces	1035210100
SalivaGene® Collector Premium	50 pieces	1035210200
SalivaGene® Collection Module II	5 container	1035212100
SalivaGene® Collection Module II	50 container	1035212200
SalivaGene® Collection Module II	125 container	1035212300
SalivaGene® Buccal Swab	5 pieces	1035230100
SalivaGene® Buccal Swab	50 pieces	1035230200
PSP® SalivaGene Precipitation Kit	50 preparations	1035290200
SalivaGene DNA Stabilizer	30 ml	1035201100
Binding Buffer A (add 21 ml)	9 ml	1035202100
Wash Buffer I (add 30 ml)	30 ml	1035203300
Wash Buffer II (add 42 ml)	18 ml	1035203400
Elution Buffer	15 ml	1035204000

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Ordering No. 6752

Applichem
2-Propanol für die Molekularbiologie
Ordering No. A3928

Sigma
2-Propanol
Ordering No. 59304-1L-F

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